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Diarrheal illness among deployed U.S. military personnel during Operation Bright Star 2001—Egypt

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Abstract

In the fall of 2001, approximately 15,000 U.S. military personnel participated in a military exercise in the northwestern Egyptian desert. To assess the prevalence and impact of diarrhea and enteropathogen distribution, we conducted a post-deployment survey and a case series study. A departure convenience sampling (n = 3725) was used in the post-deployment survey. Overall, 9.3% reported diarrhea, 2.6% sought medical care, and 2.8% stopped or decreased their work for at least a day. Among those reporting diarrhea, 41.7% had symptoms for less than 2 days, 43.5% had symptoms from 2-5 days, and 14.8% had symptoms for more than 5 days. In the case series study, pathogens were identified in 53.6% of the 129 cases enrolled. Pathogens identified included enterotoxigenic *E. coli* (n = 53), enteroaggregative *E. coli* (n = 13), *Cryptosporidium* (n = 9), *Campylobacter jejuni* (n = 7), noroviruses (n = 7), *Shigella flexneri* (n = 2), *rotavirus* (n = 2), and *Entamoeba histolytica* (n = 2). Among those seeking care for diarrhea, two thirds reported a decreased ability or inability to perform their jobs for at least one day, but overall, diarrhea was much less prevalent than in past surveys in this region, with minimal impact on the mission. Published by Elsevier Inc.

Keywords: Diarrhea; *E. coli*; Pathogens; Military; Egypt; Middle East; Cryptosporidium

1. Introduction

Traveler's diarrhea (TD) is one of the most common medical problems encountered by deployed military personnel (Connor and Farthing, 1999). A recent report showed that almost three quarters of American troops deployed to Iraq and Afghanistan experienced diarrhea, and over half had multiple episodes (Sanders et al., 2004). However, this study was based on retrospective collection of data using self-reporting surveys and thus could not ascertain pathogen distributions. To appropriately design programs to prevent and treat diarrhea in deployed troops, it is important to determine the incidence and pathogen distribution of diarrhea during military deployments. A recent military exercise conducted in the Middle East allowed for just such an opportunity.

2. Materials and methods

Operation Bright Star (OBS) is a multinational military exercise that has been conducted in Egypt in the fall of every other year since 1985. From September through November 2001, approximately 15,000 U.S. military personnel participated in OBS allowing us to conduct a diarrhea case series study among U.S. military personnel. Due to the recent occurrence of the September 11th attacks, OBS 2001 was conducted under heightened security conditions severely limiting the off-base travel of the troops and thus their exposure to locally prepared food.

In addition to the case series study, we collected a post-deployment survey assessing enteric disease among military

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participants. The survey inquired about disease experience during the entire deployment and was conducted as a convenience sampling of personnel at the time they were preparing to depart the country.

This study was approved by the Scientific Review Board and the Institutional Review Board of the U.S. Naval Medical Research Unit No. 3, Cairo, Egypt and was supported under work unit number 847705.82000.25GB.E0018_GEIS.

2.1. Clinical evaluation and specimen collection

U.S. military personnel presenting to one of two field medical clinics with diarrhea were asked to participate in the study. Diarrhea was defined as 3 or more loose or liquid stools in a 24-hour period, or 2 or more loose or liquid stools in 24 hours associated with other gastrointestinal symptoms (nausea, vomiting, cramps, tenesmus, blood in stools) or fever. Volunteers were excluded if they had participated in the study during the last five days or had used an antibiotic within 24 hours prior to clinic visit. All eligible volunteers who consented to participate underwent an extensive clinical evaluation by one of the study physicians. In addition, all study volunteers completed a detailed questionnaire, adapted from previous studies, designed to evaluate past travel, prior episodes of traveler's diarrhea, and recent exposures (Sanders et al., 2004). Diarrhea diary cards were provided to each volunteer with instructions to provide detailed information about the course of their diarrhea over the days following their primary visit. Follow-up appointments were scheduled for each study subject three days post-visit. Prior to any medical treatment, volunteers provided stool specimens for etiologic evaluation.

A field laboratory was established to process the stool specimens. For bacterial testing, stools were inoculated into Cary-Blair (CB) transport medium and sheep's blood transport medium, then stored in a cool box. For virology testing, an aliquot of stool was stored on dry ice until being transferred to -70 °C at NAMRU-3. For parasitology testing, the specimen was stored in 10% formalin and merthiolate iodine formalin (MIF). All clinical specimens were transferred to NAMRU-3 within 72 hours of collection for all subsequent testing.

2.2. Bacteriology

At NAMRU-3, standard laboratory procedures were used for the isolation and identification of major enteric bacterial species (*Shigella, Salmonella*, and *Vibrio*) (Murray et al., 2003). *Campylobacter* species were isolated on a modified Skirrow's medium (Hardy Diagnostics, Santa Maria, CA) after incubation for 48 hours at 42 °C in a microaerophilic environment (Skirrow, 1977; Saenz et al., 2000). For the Enterobacteriaceae, biochemical identification was performed (API 20E system, Analytab Products, New York, NY). Hippurate hydrolysis was used to differentiate the *Campylobacter* isolates into *Campylobacter jejuni* (hippurate positive) and non-*jejuni* (hippurate negative) (Van Looveren et al., 2001). For the identification of enterotoxigenic *E. coli* (ETEC), five individual *E. coli*-like colonies were assayed for the production of heat-labile toxin (LT) and heat-stable toxin (ST), using direct and indirect GM_1 ELISA (Svennerholm and Wiklund, 1983; Sanchez et al., 1990). For the identification of enteroaggregative *E. coli*, three randomly selected *E. coli*-like colonies were screened by three independent observers for the pattern of adherence to HEp-2 cells in the presence of D-mannose. Aggregative adherence (AA) was characterized by a stacked brick-like arrangement of bacteria on the surface of the cells, as well as on the glass cover slip free from HEp-2 cells (Cravioto et al., 1991).

2.3. Parasitology

A standard qualitative antigen detection enzyme immunoassay kit (Techlab Inc., Blacksburg, VA) was used for the identification of *Giardia lamblia*, *Cryptosporidium parvum* and *Entamoeba histolytica* from frozen stools per manufacturer's instructions. In addition, basic microscopic evaluation utilizing merthiolate-iodine-formalin (MIF) stain, as well as Ziehl-Neelsen acid fast stains, and modified trichrome stains (Corcoran et al., 1995), was performed.

2.4. Virology

Rotavirus antigen was detected using the *Rotaclone* EIA kit (Meridian Diagnostics, Cincinnati, Ohio). (Lipson et al., 2001) Astrovirus antigen was detected by ELISA kit (IDEIA from DAKO Diagnostics, Cambridgeshire, UK). (Putzker et al., 2000) Laboratory procedures for both rotavirus and astrovirus detection were performed according to manufacturer's instructions. Norovirus detection was performed using a previously described enzyme immunoassay (Jiang et al., 2000).

2.5. Statistical analysis

Data from all questionnaires and surveys were double-data entered into EpiInfo 6.0 (CDC, Atlanta, GA). All statistical analyses were conducted by SAS v8.01 (SAS Institute, Cary, NC) on exported data. To test the statistical significance among discrete variables (i.e., proportions), the Mantel-Haenszel Chi-Square or Fisher's Exact tests were used. For two-group significance testing between continuous variables, either the Student's t-test (parametic) or the Wilcoxon Rank (non-parametric) were used. Where there were more than two-group comparisons, either an ANOVA or Kruskal-Wallis were employed. Two-tail statistical significance was set at 0.05 for all analysis.

3. Results

3.1. Clinical presentation

A total of 129 patients, representing approximately 80% of personnel reporting to the clinic for diarrhea, enrolled in the study. Demographics of the volunteers can be seen in Table 1. No differences in symptoms, pathogen distribution,

Table 1
Demographics of cases (n = 129)

	Percent distributions
	N (%)
Male	100 (78.1)
Branch (N = 127)	
Army	87 (68.5)
USMC	16 (12.6)
Navy	8 (6.3)
Air Force	9 (7.1)
Civilian	7 (5.5)
Race (N = 127)	
African-American	31 (24.4)
Caucasian	81 (63.8)
Hispanic	9 (7.1)
Asian	3 (2.4)
Other	3 (2.4)
Prior Travelers' Diarrhea	
Yes	31 (24.1)
Age (yrs)	
Mean	31.0
Median	29.5
Range	19 – 63
Time in country prior to enrollment (days)	
Mean	23.5
Median	23
Range	1 – 58

or treatment were detected based on age, rank, or gender. The median number of loose or liquid stools in the 24 hours preceding the initial clinic visit was 4, with an interquartile range (IQR) of 3 – 7 stools. The median number of loose or liquid stools reported during the entire duration of illness prior to presentation was 9 (IQR, 4 – 15) stools. The overall median time from the start of symptoms to treatment was 37 hours (IQR = 12 – 62). Only two cases (1.6%) reported having a history of bloody stools. Associated symptoms are displayed in Table 2. At the time of initial evaluation, 13.4% reported 'not being able' to work due to their symptoms. Additionally, over half (54%) of the participants reported a decreased ability to perform their duties.

3.2. Pathogens

Of all the cases (n = 129) enrolled during this study, 75 (58%) had one or more enteric pathogens recovered from their stool specimen for a total of 94 pathogens (Table 3).

Table 2
Clinical parameters reported during initial clinic visit (n = 128)

	N (%)	Median (IQR) duration (Days)
Diarrhea	128 (100)	1.0 (1 – 3)
Cramps	116 (91)	1.0 (1 – 2)
Nausea	78 (61)	1.0 (1 – 2)
Headache	54 (42)	1.0 (1 – 2)
Muscle Ache	38 (30)	1.0 (1 – 2)
Subjective Fever	32 (25)	1.0 (1 – 1)
Tenesmus	31 (24)	1.0 (1 – 3)
Vomiting	21 (17)	1.0 (1 – 1)
Joint Pain	9 (7)	1.0 (1 – 2)
Bloody stools	2 (2)	NA

Fifty-nine of the cases had a single pathogen recovered, while 16 cases had two or more pathogens isolated. The most common pathogens identified were ETEC (n = 52) and EAEC (n = 13). Enteric parasites were recovered from 11 cases (4 solo infections; 7 mixed infections). The most common parasite identified was *Cryptosporidium parvum* (n = 9), which was frequently identified as a co-pathogen in mixed infections (n = 7). A total of nine cases (7%) had enteric viruses identified. The most common viruses identified were the noroviruses, which were identified in seven cases, while the majority of enteric viruses recovered were co-pathogens from mixed infections.

3.3. Medical treatment

Of the 129 cases, 125 cases received some form of medical therapy and follow-up was completed for 88 (68%). Of those that were treated, 92% of the cases were cured within 72 hours, and only 5 cases reported a relapse of their symptoms. The most common therapy administered was a combination of loperamide and levofloxacin (54.3%), with loperamide and ciprofloxacin next (28.7%), followed by loperamide alone (12.5%) (Table 4). Comparing the median duration of post-treatment symptoms among cases with a single bacterial pathogen, there was no statistically significant difference noted between levofloxacin/loperamide versus ciprofloxacin/loperamide (14.9 hours vs. 15.9 hours, respectively; $P = 0.8$). In addition, there was no statistically significant difference between ETEC or EAEC with respect to post-treatment symptoms. Statistical analysis of other bacterial causes of diarrhea were not possible due to the limited number of cases. Cases of non-bacterial diarrhea were more prolonged than cases from which a bacterial pathogen was isolated from the stool. Prolonged symptoms were particularly prominent for

Table 3
Pathogen distribution among all diarrhea cases (n = 129) collected during Operation Bright Star

	Sole pathogen n (%)	Mixed infection n (%)	Total
Bacteria			
ETEC[a]	41 (32%)	11 (9%)	52
EAEC[b]	9 (7.0%)	4 (3.1%)	13
Campylobacter sp.	2 (1.6%)	5 (3.9%)	7
Shigella sp.	1 (0.8%)	1 (0.8%)	2
Parasite			
C. parvum	2 (1.6%)	7 (5.4%)	9
E. histolytica	2 (1.6%)	0	2
G. *lamblia*	0	0	0
Virus			
Rotavirus	0	2 (1.6%)	2
Astrovirus	0	0	0
Norovirus	2 (1.6%)	5 (3.9%)	7
No Enteric Pathogen Identified	54 (42%)		54

[a] Enterotoxigenic *Escherichia coli*.
[b] Enteroaggregative *Escherichia coli*.

Table 4
Frequency distribution and MEDIAN duration (hours) of diarrheal symptoms before and after treatment among cases with a single pathogen recovered, stratified by pathogen type

	Bacterial				Parasitic			Viral			
	ETEC	EAEC	SHIG	CAMPY	CRYPTO	EHISTO	GIARDIA	ROTA	NORO	ASTRO	No pathogen
	Pre-therapy symptoms duration (hours)										
Cases	52	13	2	7	9	2	–	2	2	–	52
Median (hrs)	39.3	43.0	41.5	24.2	48.3	48.9	–	61.4	65.0	–	24.1
	Post-therapy symptoms duration (hours)										
Cases	38	11	2	5	7	2	–	1	5	–	38
Median (hrs)	8.6	18.4	1.8	10.8	41.0	9.1	–	2.0	4.0	–	18.8
	Post-therapy symptoms duration (hours/(N)) stratified by treatment modality										
I[a]	14.8 (2)	26.5 (1)	–	–	–	–	–	–	–	–	21.2 (4)
C & I[b]	7.3 (14)	14.3 (4)	1.8 (1)	–	–	9.0 (1)	–	–	–	–	27.7 (10)
L & I[c]	9.5 (13)	17.8 (2)	–	10.8 (1)	21.8 (1)	9.3 (1)	–	–	9.5 (2)	–	5.8 (20)

[a] Imodium alone.
[b] Ciprofloxacin & Imodium.
[c] Levofloxacin & Imodium.

Cryptosporidium-associated diarrhea with patients being ill an median of 41 hours.

Of the clinical failures, 38% reported missing one or more days of work, while 20% of the clinical successes reported missing one or more days of work ($P = 0.3$). Overall, the mean number of days reported as 'missed' by cases during the follow-up exam was 0.3 days (range 0 – 3).

Inclusion of an antimicrobial agent in the treatment regimen did not appear to to be associated with functional ability at presentation: normal ability (82.5%), decreased ability (88.6%), and not able to function (82.0%) ($P = 0.6$). However, there was a significant difference found comparing post-treatment duration of diarrhea and functional ability at presentation with respect to pathogen recovered (Table 5) and the specific pathogen type subsequently identified (Table 6).

3.4. Risk associations

To assess possible food/drink exposure, recent (within five days of case-episode) food/drink consumption data was collected from all volunteers who reported to the clinic with diarrhea. Even with increased security, local food consumption was common among personnel with diarrhea, as 73% of the cases reported consuming food/drink from both U.S. military food services and local Egyptian sources, but there was no statistical association for acquiring ETEC-associated diarrhea relative to dining facilities ($P = 0.3$). There was a statistically significant increased risk for ETEC-associated diarrhea noted for those cases in which fresh fruit ($P = 0.04$), fresh vegetables ($P = 0.003$), seafood ($P = 0.03$) and fish ($P = 0.02$) were consumed. Risk association for other enteric pathogens could not be conducted due to small numbers in each category.

3.5. Post-deployment survey

A total of 3725 U.S. military personnel completed a post-deployment survey. Of these, 348 (9.3%) reported an episode of diarrhea at some point during the military exercise. Among the respondents with diarrhea, 63 (18%) reported fever in concurrence with diarrhea during the deployment. The survey found that 104 (30%) of those with diarrhea reported missing at least one day of work. The duration of diarrhea was reported as a categorical variable with the following proportions: 135 (39%) reported a duration of less than 2 days, 141 (41%) reported a duration of 2 – 5 days, and 48 (14%) reported a duration of greater than 5 days. Ten percent of respondents (388) reported bringing medicines from home to treat diarrhea, and of these, 22% (85) developed diarrhea. Of the 348 respondents reporting having diarrhea, 98 (25%) sought medical attention.

Table 5
Duration of post-treatment diarrhea based on subsequent pathogen identification

	Any pathogen identified		No pathogen identified	
	N	Duration (median, IRQ)[a]	N	Duration (median, IQR)[a]
Normal	17	10.8 (3.0 – 26.5)	16	16.8 (1.2 – 24.8)
Decreased	36	9.4 (3.4 – 28.9)	15	7.5 (1.0 – 31.2)
Not able	6	9.1 (3.0 – 26.5)	4	39.3 (25.6 – 77.0)*

[a] Hours (Interquartile Range [IQR]).
* $P < 0.05$.

Table 6
Functional ability at presentation, pathogen type recovered and duration of post-treatment diarrhea

Functional ability	Duration of diarrhea post treatment[b]	Pathogen distribution[a]		
		Bacterial	Parasitic	Viral
Normal	15.5 (2.0 – 25.1)	17 (28%)	0	0
Decreased	9.3 (1.8 – 29.2)	35 (59%)	1 (25%)	2 (100%)
Not able	21.5 (9.0 – 44.0)	7 (12%)	3 (75%)	0

[a] Number.
[b] Hours (Interquartile Range [IQR]).

4. Discussion

The objective of this study was to assess diarrheal disease among U.S. military personnel deployed in Southwest Asia. We found that diarrhea continues to contribute to the overall morbidity of deployed U.S. military personnel, possibly reducing operational effectiveness. While the occurrence of diarrhea was much lower than historical reports from surveillance studies conducted in conjunction with previous OBS operations, (Haberberger et al., 1991; Oyofo et al., 1995) approximately 9% of troops reported having at least one episode of diarrhea.

This low occurrence is likely due to the extreme travel restrictions placed on the troops due to the temporal proximity to the September 11th attacks. Unlike previous OBS where troops had the opportunity to visit Egyptian tourist sites, in OBS 2001 troops were essentially kept on base with the exception of traveling to and from the airport. Since eating local Egyptian food and visiting local tourist areas have been identified as the major risk factors for developing diarrhea, (Sanchez et al., 1998) this lack of access may have resulted in decreased diarrhea rates. This rate is significantly lower than the post-deployment, self-reported rates from past exercises in Egypt: 1983 (40.3%), 1987 (40%), and 1989 (19.1%) (Haberberger et al., 1991; Sanchez et al., 1998).

ETEC was the most common pathogen isolated in this study. While ETEC is the most common cause of travelers' diarrhea worldwide, it usually causes a milder illness than others such as *Campylobacter* or *Shigella* (Mattila, 1994; Sanders et al., 2002). Despite the relatively mild symptoms, over half of participants in this study with diarrhea reported a decreased ability to perform their jobs. These data suggest that additional studies need to be performed to assess the operational impact of diarrhea, specifically ETEC-associated diarrhea, in deployed U.S. military personnel.

Cryptosporidium was the third most common pathogen isolated in this study. This is not surprising given the frequency with which *Cryptosporidium* has been identified from pediatric diarrhea cases among residents in the Nile River Delta region in Egypt (Abdel-Messih et al., (in press)). The majority of *Cryptosporidium* cases in this study were part of mixed infections, so it is difficult to assess the specific clinical symptoms and operational impact of this organism. However, symptoms associated with this infection lasted significantly longer (41.0 vs. 9.4 hours; $P = 0.008$) following treatment than with any other identified pathogen. Therefore, it remains unclear whether *Cryptosporidium* should be considered a significant pathogen in deployed military personnel and additional studies need to be conducted.

If self-described functional ability could be associated with certain infections, it potentially could be used as a criterion for determining treatment regimens. With respect to functional ability, personnel who described themselves as having a normal ability were equally likely to have an identified pathogen or no identified pathogen. Personnel who reported that they had a decreased ability to work were more likely to have an identified bacterial pathogen. A parasitic infection was more likely to be identified in those personnel stating that they had an inability to work, but whether they had a bacterial or parasitic infection, they had significant time to cure benefit from treatment if a pathogen was identified. The group that apparently did not benefit as much from treatment was the group that had an inability to work, but no pathogen identified, suggesting either an unidentified parasite or resistant bacteria or non-infectious cause (irritable bowel syndrome, celiac disease, etc.).

5. Limitations

The dispersion of troops over a large geographical area along with the limited number of study personnel required the implementation of a passive surveillance network. Therefore, patients were only able to be enrolled in the study when they reported to a clinic for medical care, so there is the possibility of a skewed pathogen distribution toward the more pathogenic (severity and length of disease) organisms among the people who chose to seek medical attention. Based on the post-deployment survey, only 2.8% of all personnel sought medical care at a clinic. Another possible limitation of our study was the loss of pathogen(s) during specimen transport. However, a study demonstrated that isolation of pathogens remained high from Cary-Blair transport medium even up to two weeks later (Wasfy et al., 1995). The post-deployment survey also was potentially influenced by the collection design. Due to the frequent changes in schedules, we were unable to conduct either a systematic or random sampling of departing U.S. military personnel. We therefore were forced to perform the post-deployment survey using a convenience sampling, which may have biased diarrhea estimates. Nevertheless, since the survey was distributed over several weeks and nearly a quarter of the deployed personnel completed it, the chances of selection bias should be minimized.

6. Conclusion

Among those personnel who reported to clinic for diarrhea, two-thirds reported a decreased ability or inability to perform their job. Additionally, post-deployment survey results demonstrated that even with the best of preventive medicine efforts almost ten percent of the troops experienced diarrhea at some time during the deployment. These data may indicate that passive surveillance systems significantly underestimate the true incidence of diarrhea. ETEC and EAEC were the most common pathogens isolated, but continued epidemiologic studies among deployed military personnel are necessary to assess the significance of other pathogens, such as *Cryptosporidium* and norovirus, and to

determine the operational impact of acquired diarrhea disease on military readiness under other deployment conditions.

Author's contributions

Study Concept/Design: Sanders, Frenck
Acquisition of Data: Sanders, Gould Kolisnyk, Merced, Barthel
Laboratory Determinations Rozmajzl, Shaheen., Fouad,
Interpretation: Sanders, Putnam, Frenck
Drafting manuscript: Sanders, Putnam
Critical revision of the manuscript for important intellectual content: Sanders, Putnam, Frenck
Statistical expertise: Putnam
Obtained funding: Sanders, Frenck,
Study supervisors: Sanders, Frenck

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